Insulin Response to Glucose or Glucagon in Subclinical Diabetes Previously Injected with Tolbutamide

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Thirty-one patients with subclinical diabetes, who showed diabetic or impaired glucose tolerance after treatment for diabetes, were investigated in order to clarify the abnormalities of insulin response in diabetes mellitus. These patients showed a delayed response of plasma insulin during oral glucose loading. In the tolbutamide-glucose test, in which glucose loading followed the intravenous tolbutamide injection at a 60-min interval, the insulin level at 90 min was significantly lowered in a group of 20 patients with subclinical diabetes. In the tolbutamide-glucagon test, in which 1 mg of glucagon was injected 60 min after tolbutamide injection, the maximal level of plasma insulin was significantly decreased in a group of 10 subclinical diabetics except for one patient. These results indicate that insulinogenesis and/or release of insulin were decreased even in subclinical diabetes, suggesting that such a defect in islet function might be one of the abnormalities in primary diabetes.

Subclinical diabetes; insulin response; tolbutamide; glucose tolerance; glucagon test

In order to investigate insulin response in diabetes mellitus, the glucose loading test has been widely employed. However, as reported previously (Ohneda et al. 1974), it is usually very difficult to discriminate diabetes mellitus from the normal condition in view of insulin response. In 1974, we reported that the plasma insulin level 30 min after glucose load was significantly decreased in diabetes mellitus compared with the normal controls when glucose was given 60 min after tolbutamide administration (Ohneda et al. 1974). Furthermore, a decrease in the maximal insulin response was observed in diabetes mellitus when glucagon was administered 60 min after tolbutamide injection (Ohneda et al. 1975b). Therefore, in this study tolbutamide-glucose loading and tolbutamide-glucagon test were carried out in subclinical diabetes, to see if decreased response of plasma insulin can be detectable as observed in overt diabetes.

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SUBJECTS AND METHODS

In this study, 31 patients were investigated. These patients were selected as subclinical diabetes among patients who showed diabetic glucose tolerance and treated with diet alone for one to eleven years. Therefore, subclinical diabetes implies patients with diabetic glucose tolerance or decreased glucose tolerance and with the fasting blood glucose level below 120 mg/100 ml. The standard glucose tolerance test (GTT) was carried out in 31 subclinical diabetes after an overnight fast by the method described previously using 50 g glucose (Goto et al. 1960). In 21 of subclinical diabetes, tolbutamide-glucose loading (TGTT) was performed by the method stated elsewhere (Ohneda et al. 1974). In the other 10 patients, tolbutamide-glucagon loading (TGT) was carried out according to the method previously reported (Ohneda et al. 1975b). For the comparison, the data for the normal controls and overt diabetes were quoted from the results in GTT, TGTT or TGT (Ohneda et al. 1974, 1975a, b).

Capillary blood was obtained from the ear lobe and glucose was measured by the glucose oxidase method (Teller 1956). Blood for insulin assay was drawn from the antecubital vein and plasma was separated by centrifugation. Plasma was kept at -20°C until the assay began. Plasma insulin was determined by the Morgan-Lazarow method (1962). The mean values and the standard error of mean were calculated and statistical analyses were performed by Student’s t-test.

Results

Glucose tolerance test

The mean blood glucose and plasma insulin during GTT in 31 subclinical
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diabetics are presented in Fig. 1, with the results of the normal controls (Ohneda et al. 1975 a). In the subclinical diabetics, the blood glucose level rose from the base line of 100.3±2.4 mg/100 ml to a peak of 209.5±7.7 mg/100 ml at 60 min and remained at the level of 160.4±9.5 mg/100 ml at 120 min. 18 of 31 subclinical diabetics revealed diabetic glucose tolerance curves, as classified by the recommendation of Japan Diabetic Society on the criteria of diagnosis for diabetes mellitus (Kuzuya 1970). Plasma insulin increased from the initial level of 14.6±1.6 μU/ml to a peak of 69.4±6.7 μU/ml 60 min after glucose load. The mean insulin levels at 60, 90 and 120 min in the patients were significantly higher than that in the controls (p<0.001). 22 patients showed a retarded pattern of plasma insulin response (low early, high later rise), while 7 patients revealed a normal pattern of insulin response.

Tolbutamide-glucose loading

The changes in blood glucose and plasma insulin are presented in Fig. 2, where the results for 8 normal controls and 8 patients with severe diabetes are depicted for the comparison (Ohneda et al. 1974). In subclinical diabetes, blood glucose fell from the initial level of 102.0±3.2 mg/100 ml to a nadir of 63.7±2.8 mg/100 ml 60 min after tolbutamide injection. After glucose ingestion at 60 min, blood glucose rose to a peak of 209.5±7.7 mg/100 ml at 60 min and remained at the level of 160.4±9.5 mg/100 ml at 120 min. The shaded areas indicate normal ranges.

**Fig. 2.** Changes in blood glucose and plasma insulin during tolbutamide-glucose load in severe and subclinical diabetics. •—•, subclinical diabetes (N=21); ○—○, severe diabetes (N=8). The shaded areas indicate normal ranges.
glucose rose to a peak of 164.9±6.4 mg/100 ml at 120 min and then fell. Therefore, slower decrease in blood glucose after tolbutamide and sustained hyperglycemia during glucose load may be the commonest feature in TGTT for subclinical diabetes. Plasma insulin rose from the fasting level of 14.3±2.8 μU/ml to a peak of 40.6±5.4 μU/ml 10 min after tolbutamide and the mean levels of maximal plasma insulin after tolbutamide were within the normal range. After glucose ingestion plasma insulin rose slowly and the values at 120 through 180 min were within the normal range. However, the insulin levels at 90 min was significantly decreased in comparison with the normal controls (p<0.01). On the other hand, insulin response in the group of 8 severe diabetics was minimum after tolbutamide as well as after glucose.

**Tolbutamide-glucaigon load**

Changes in blood glucose and plasma insulin are summarized in Fig. 3. In addition to the results for 10 subclinical diabetics, those for the normal controls and 3 patients with moderate diabetes are presented in the figure. Blood glucose decreased slowly after tolbutamide injection to a nadir of 71.8±4.4 mg/100 ml at 60 min in subclinical diabetes. After glucagon was injected, blood glucose rose abruptly and reached a peak of 133.8±3.0 mg/100 ml, falling to the preinjection level at 180 min. The patients with moderate diabetes showed a

![Fig. 3. Changes in blood glucose and plasma insulin during tolbutamide-glucaigon test in moderate and subclinical diabetics.](image-url)

- ●▬●, subclinical diabetes (N=10); ○▬○, moderate diabetes (N=3).
  The shaded areas indicate normal ranges.
pattern of changes in blood glucose similar to that of subclinical diabetes. The plasma insulin level rose from the fasting level of $10.7 \pm 2.6 \mu U/ml$ to a peak of $41.1 \pm 7.3 \mu U/ml$ 6 min after tolbutamide injection in subclinical diabetes. Following glucagon injection plasma insulin reached a peak of $52.7 \pm 11.9 \mu U/ml$ at 10 min and then fell to the preinjection level by 180 min. In the moderate diabetics, the maximal levels of plasma insulin after tolbutamide and glucagon were $8.8 \pm 3.4$ and $27.7 \pm 10.3 \mu U/ml$, respectively. Therefore, the responses of plasma insulin to tolbutamide or glucagon in subclinical diabetes were just at the midway between the normal controls and the moderate diabetics. There was no significant difference between the maximal insulin response to glucagon in the subclinical diabetics and the normal controls because of wide deviation due to a patient with exceptionally elevated plasma insulin. Therefore, when the patient was excluded, the maximal insulin response to glucagon in the subclinical diabetics was significantly decreased compared with the controls ($p<0.001$).

**DISCUSSION**

In the present experiment, 31 patients with diabetic or impaired glucose tolerance were selected from the patients who had been treated with diet alone for one to eleven years. Therefore, patients with a transient diabetic glucose tolerance curve might be excluded. Although a few patients in the original group developed to overt diabetes during the observation period, 31 patients could be regarded as those who were at the state of subclinical diabetes of primary diabetes. Therefore, information obtained from these patients as to insulin response may contribute to the investigation of the endocrine function of the pancreas in diabetes mellitus.

In the study, there was no decreased response of plasma insulin during GTT in subclinical diabetes compared with the normal controls. In fact, the mean levels of plasma insulin during GTT in the subclinical diabetics were significantly higher at 60 through 120 min than in the normal subjects. To the contrary, both the insulin levels at 90 min in TGTT and the maximal response of plasma insulin to glucagon in TGTT were decreased in the subclinical diabetics. Furthermore, the 90-min insulin levels during TGTT were significantly lowered in the patients with severe diabetes compared with the normal controls. This was true in the case of the maximal insulin response to glucagon in TGTT. These results indicate that the decreased insulin response can be demonstrated even in the subclinical diabetics during TGTT or TGTT.

Since significantly decreased responses were observed in plasma insulin during TGTT or TGTT, but not during GTT, pretreatment with tolbutamide may magnify the decreased insulin response in diabetes mellitus. As tolbutamide seems to stimulate the release of stored insulin in the B cells of the pancreatic islets (Grodsky et al. 1968), successive administration of glucose or glucagon might induce insulino-genesis and/or the release of newly synthesized insulin in the B cells. In the normal controls, insulin levels at 90 min during TGTT were higher than those at 30
min during GTT (Ohneda et al. 1974). In contrast, insulin response to glucose or glucagon in subclinical diabetes was not exaggerated by the previous injection of tolbutamide but rather decreased. Therefore, the decreased response of plasma insulin to glucose or glucagon during TGT or TGT in the subclinical diabetics supports the previous conclusion that insulinogenesis in the B cells of the islets is, in general, decreased in diabetes mellitus (Ohneda et al. 1974).

The patients with subclinical diabetes studied in the experiment had been treated with diet, in contrast to the previous report in which diabetic patients were investigated before diabetic treatment (Ohneda et al. 1974). Nevertheless, the patients in this study revealed diabetic glucose tolerance or impaired glucose tolerance as well as decreased response of plasma insulin during TGT or TGT. These results suggest that the decrease in insulinogenesis and/or the release of newly synthesized insulin in the B cells would be one of the primary abnormalities in diabetes mellitus.

References